Safer food through rapid tests for chemical contaminants

Philippe Vermeulen and Jacob de Jong give an overview on the aims and progresses of the new EU FP7 CON*ff*IDENCE project.

The CON*ff*IDENCE project

RASFF, the rapid alert system on food and feed shows that controls on chemical contaminants in food and feed are essential for food safety in Europe. Safer food through rapid and cost-efficient tests for detecting chemical contaminants in food and animal feed is the major goal of a new four-year European research project called CON*ff*IDENCE. This project launched in May 2008 is entitled ”Contaminants in Food and Feed: Inexpensive Detection for Control of Exposure”. It is coordinated by Dr Jacob de Jong from RIKILT – Institute of Food Safety, part of Wageningen UR, The Netherlands. The CON*ff*IDENCE consortium consists of 17 partners from 10 European countries.

One of the major concerns of European governments and consumers is the presence of chemical contaminants in food. Consequently, Regulatory Authorities and the food/ feed industries spend large budgets to monitor and control the safety of both food products and animal feed. This monitoring often uses expensive methods that can only detect one specific chemical. There is therefore an urgent need for replacement of current methods by validated screening tools, which are simple, inexpensive and rapid and are able to detect as many chemical contaminants in parallel as possible.

The CON*ff*IDENCE project has been designed to provide long-term solutions to the monitoring of a wide variety of chemical contaminants. These include persistent organic pollutants (POPs), perfluorinated compounds (PFCs), pesticides, veterinary pharmaceuticals such as coccidiostats or antibiotics, heavy metals and biotoxins such as alkaloids, marine toxins or mycotoxins. Tests will be developed and validated for products such as fish and fish feed, cereal-based food/feed and vegetables. A balanced mix of novel multiplex technologies will be utilized. These include dipstick tests that can be used in the same way as pregnancy tests, and low-cost laboratory-based high-throughput methods.

After completion, the simplified methods will be used to perform international food surveys that will contribute to measurement of consumer exposure to chemical contaminants. Moreover, the tests will be used to study the transfer of harmful contaminants from feed to eggs and meat.

The first annual meeting, hosted by Consejo Superior de Investigaciones Cientificas (CSIC), was held on the 5th and 6th of March 2009 in Barcelona. It was attended by

more than 45 researchers from all 17 CONffIDENCE partners (Figure 1). During this meeting the work package leaders presented the first year activities and achievements in the nine RTD work packages gathered in 4 clusters: organic pollutants, veterinary pharmaceuticals, heavy metals and biotoxins.

Figure 1. Participants to the 1st annual meeting.

The organic pollutants

The **organic pollutants cluster** led by Marinella Farre (Consejo Superior de Investigaciones Cientificas, Spain) is dedicated to study persistent organic pollutants (POPs), perfluorinated compounds (PFCs) and pesticides.

Regarding the **POPs**, the main goal is to develop a flow cytometry based multiplex assay for bioactive POP metabolites using transport proteins and antibodies as biorecognition elements in fish, fish feed and cereal-based baby food. A comprehensive metabolomics-like profiling strategy for POPs, including simplified sample preparation will be designed as well.

Regarding the **PFCs**, a simplified and harmonized analytical procedure for the detection and quantification of Perfluorooctanesulfonate (PFOS), perfluoro octane sulfonamide (FOSA), and perfluorooctanoic acid (PFOA) in food has been setup. A method based on the use of activated charcoal for extraction and clean up, followed with liquid chromatography and mass spectrometry has been developed. The method presents simple and rapid extraction/clean up steps and the use of commonly chromatographic equipment in routine laboratories.

Regarding the **pesticides**, the objectives are to validate an electrochemical magneto immunosensor assay for the single residues paraquat and diquat in potato and cereals and to carry out a feasibility study on the use of DESI/DART as a high-throughput simplified mass spectrometric screening tool for the single residue issue of dithiocarbamates on intact vegetables.

The veterinary pharmaceuticals

The **veterinary pharmaceuticals cluster** led by Sara Stead (Food and Environment Research Agency, United Kingdom) aims to develop rapid and low cost screening strategies for detection of veterinary drug residues in foods of animal origin. The new

assays will be validated in accordance with the current EU guidelines (2002/657/EC) and used to perform small-scale predictive hazard modeling studies.
One focus is on the development of a multiplexed screening assay for the detection of **coccidiostats**. Coccidiostat agents constitute the main choice to fight coccidiosis, a major parasitic disease in poultry. Recently the Commission requested an opinion of EFSA on the risks involved for animal and public health as a consequence of unavoidable cross-contamination of frequently used coccidiostats authorised as feed additive into non-target feeds and consequently the presence of such residues in food of animal origin. The project will address the priority coccidiostats lasalocid, monensin, diclazuril, narasin, nicrabazin and salinomycin in laying hens feed produced on the same production line as broiler feed containing the coccidiostats at the normal therapeutic dose and consider both eggs and feed as sample matrices of interest. Two main tasks were addressed during the first project year, namely the production of all relevant immunochemical reagents (antibodies and conjugates) and the production of egg and feed test materials. The antibodies produced were characterized by ELISA before being tested in beads coupling experiments for the LuminexTM multiplex assays. The characterization of all feed and some egg test materials is currently on going.
A second focus in this cluster is on the development of low-cost, portable screening methods based on dipstick technology for the detection of **antibiotics** that can be used by inspection officials. In 2005/2006 ,65% of the 300 RASFF notifications for drugs in foods of animal origin related to antibiotics. The major problem areas included malachite green in fish, tetracycline usage and multiple antibiotics in imported honey. Malachite green (MG) is not listed as a veterinary medicine but is frequently detected as an unauthorised product in fish. An MRPL of 2 $\mu g\ kg^{-1}$ has been established in 2004/25/EC due to the carcinogenicity of MG. Typically 60% of the antibiotics administered to farm animals within Europe are tetracyclines. A rapid assay for monitoring low-level tetracycline residues is required to assess the effect of consumer exposure to chronic sub-MRL concentrations, in terms of the emergence of tetracycline resistant bacteria in the human population. The issue for honey is the presence of multiple antibiotic residues of which chloramphenicol, tylosin and sulfonamides being among the most commonly reported. The use of antibiotics in apiculture is not permitted under EU legislation. In 2003/181/EC an MRPL of 0.3 $\mu g\ kg^{-1}$ for chloramphenicol in honey has been established. More recently, residues of fluoroquinolones (enrofloxacin, ciprofloxacin and norfloxacin) have been detected in Chinese honey. A 4-probe dipstick will be developed incorporating biological recognition elements to detect the presence of multiple antibiotics in honey.
During the first project year a range of test materials containing the target antibiotics have been produced for assay development and validation purposes. Based on the current antibiotic usage trends a range of matrix/ analyte combinations have been produced at concentrations close to, and above the EU MRL/MRPL concentrations (where appropriate). The test materials have been characterized using mass spectrometric confirmatory analytical methods and are subject to on-going stability monitoring at regular intervals. During the last 6 months work has been conducted to extend the scope of application for the Tetrasensor® assay. The Tetrasensor® is a commercially available competitive receptor based dipstick assay for the class specific detection of tetracycline compounds present at (and around) MRL concentrations in different matrices such as milk, honey and raw animal tissue developed by partner, Unisensor. The assay has been modified to detect target tetracycline compounds including both natural and semi-synthetic analogues;

tetracycline (TC), doxcycline (DOX), oxytetracycline (OTC) and chlortetracycline (CTC) in animal feeds, urine and cooked meat in accordance with the legislative limits (where applicable). The overall time required for the sample preparation and analysis is currently 15 minutes. The detection capability for the tetracycline compounds in raw muscle tissue is less than half the EU MRL of 100 μg kg^{-1} and circa 100 μg kg^{-1}in feeds and urine. The Tetrasensor® assay has also been optimised for compatibility with cooked meat. Tetracyclines are thermally unstable and their residue concentrations are significantly reduced following the cooking process. The optimised assay sensitivity is good and can detect the most thermally resistant tetracycline, DOX at 10-15 μg kg^{-1} in the cooked pig muscle test material (Figure 2).

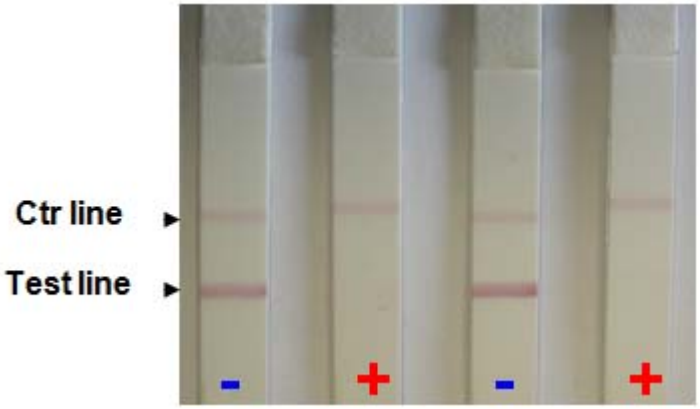

Figure 2. Photograph of the results of the Tetrasensor® analysis of the cooked pig muscle test material containing doxcycline (15 μg kg^{-1}). The control (Ctr) and test lines are visible for the DOX test material (+) and a negative control (-) sample, analysed in duplicate.

The heavy metals

The total contents of the heavy metals lead, cadmium and mercury in food and feed are regulated by (EC) Nos 466/2001/EC, 78/2005/EC, 2002/32/EC and 2003/100/EC. However, for some heavy metals the chemical form (i.e. their speciation) is important in terms of food and feed safety. While for arsenic the inorganic forms are the most toxic and for mercury, methylmercury is the most toxic form, the **heavy metal cluster**, led by Jens J. Sloth (National Food Institute, Denmark) focus on the development of simplified and inexpensive methods for these analytes. Since seafood is the major dietary source for both arsenic and mercury in the European population, the project focus on marine feed and seafood as sample matrices of interest. Two parallel approaches are being pursued. The first is a cytosensor approach using luminescent recombinant bacterial cell biosensors (CYT). The cytosensor contains a reporter gene under a highly specific inorganic or organic metal species responsive element of interest. Such a novel assay will measure only the bioactive fraction available to the living cells and set a new standard for metal speciation analysis in food and feed.Biosensors for mercury (total and inorganic form) and inorganic arsenic species are ready and tested with ionic forms of these metals or metalloids. The lowest limit of detection for the mercury sensors is 2 nM and for arsenic species (III and V) 300 nM. The freeze-drying of the sensor cells for permanent storage and future reagent-like usage is presently going on. This allows the testing of the sensors in collaborative trials. The second approach is a solid phase extraction followed by atomic absorption spectrometry (SPE-AAS). Until now an improved extraction

approach for inorganic arsenic has been developed using an acidic extractant solution and microwave oven technology. An added benefit is the addition of hydrogen peroxide to the solution converts As(III) to As(V) thus allowing for the determination of total inorganic arsenic as As(V). A commercial available SPE cartridge has been found and a method for selective elution of inorganic arsenic has been developed for standard solutions and is currently being tested on real marine samples. In the future the SPE-AAS method will be in-house validated and subsequently tested in a collaborative trial.

The biotoxins

The **biotoxin cluster** led by Chris Elliott (Queen's University Belfast, United Kingdom) concerns studies on alkaloids, marine biotoxins and mycotoxins. For each class of biotoxins, target toxins and products have been identified, and a strategy for development of improved, rapid detection methods has been established using multiplex dipticks.

Regarding the **alkaloids**, three major groups are studied, pyrrolizidine alkaloids (PA), tropane alkaloids (TA) and ergot alkaloids (EA). For all three groups EFSA scientific opinions have been prepared (EA, TA and PA in feed) or are requested (EA in food). PA are an emerging issue and found in many plant genera and, consequently, in feed, food and herbs. Matrices of primary interest are honey and feed. TA are mainly found in feed as contaminants from Datura species. Plants producing TA have expanded dramatically in parts of Europe and problems are emerging. The EFSA opinion on EA in feed shows that there is a lack of data on EA patterns in feed materials and on toxic effects. Matrices of interest are feed and cereals. The current limit in the EU is 0.1% for rye ergot in all feedingstuffs. The Commission is preparing regulations for EA in food and feed, and there is a clear need for fast and reliable methods of analysis. For this purpose near infrared imaging is investigated as well to detect ergot bodies in food and feed.

Marine toxins are a serious food safety issue, of great relevance to the European consumer. The toxins are produced in algae and accumulate in filter feeding animals such as shellfish. For as long as this biotoxin problem has been known the means of detecting them has been the use of the mouse bioassay, a procedure which involves the administration of shellfish extracts to experimental mice to determine if there are toxins present. Though this procedure has served to protect the public it is a method that needs urgently replaced. There are several significant drawbacks associated with this method: insufficient sensitivity, expensive (need of animal house), very slow (24 hours observation time, plus extraction time) and substantial ethical concerns. In addition to the problem of the mouse bioassay, there are other problems that complicate the situation in relation to marine toxin monitoring. The emerging issues related to marine toxins are the climate change modifying the toxin profile in Europe, the understanding on how many of the toxin present in shellfish extracts that kill or harm the mice are actually a human problem, the insufficient information with regards to the newly emerging toxins. As a consequence of the importance of marine toxins to the safety of the consumer large scale efforts are being placed into developing alternative methods to the mouse bioassay. The CON*ff*IDENCE project aims to produce a chip based technique which will be capable of detecting all the important marine toxins.

Fungi of the genus Fusarium are common plant pathogens occurring worldwide, mainly associated with cereal crops. The Fusarium **mycotoxins** have been implicated as the causative agents in a variety of animal and human diseases. They are widely

distributed in the food chain. The major sources of dietary intake of Fusarium toxins in Europe are cereal based foods, mainly derived from wheat and maize. The Scientific Committee on Food has established tolerable daily intakes (TDI) for DON, ZEA, total fumonisins, and the sum of T-2 and HT-2 toxins. Maximum limits for DON, ZEA and fumonisins in cereals and cereal based food products have been set, and guidance values for feed have been established as well. In the CON*ff*IDENCE project, multiplex screening assays will be developed addressing fumonisins (FB1, FB2), deoxynivalenol (DON), T-2 and HT-2 toxins, and zearalenone (ZEA) in feed, cereals and cereal based food.

Further information …

Dissemination to scientists and to relevant stakeholders, including the food and feed industry, regulatory control (DG-SANCO, EFSA) and normalisation bodies (CEN), the Community reference laboratories (CRLs), the routine laboratories and the consumers is assured by a website, an electronic newsletter and press articles. In the next years, public workshops, open days and training modules will be organised. Lectures at international conferences and publications will provide you scientific information on the project results. Let you inform by visiting regularly the website http://www.conffidence.eu. Those dissemination activities are led by Dr Vincent Baeten and Philippe Vermeulen (Walloon Agricultural Research Centre, Belgium) in collaboration with the coordination team, Jacob De Jong, Stefan Weigel, Lonneke van der Geest and Wim Beek (RIKILT, The Netherlands).
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Acknowledgements:
The research leading to these results has received funding from the European Community's Seventh Framework Programme (*FP7/2007-2013)* under *grant agreement* n° 211326.

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